HIPPOCAMPAL DENDRITIC SPINES ARE RESISTANT TO THE DETRIMENTAL EFFECTS OF STRESS DURING MOTHERHOOD

Honors Research Thesis

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By

Aarthi Gobinath

The Ohio State University

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Project Adviser: Dr. Benedetta Leuner, Department of Psychology

ABSTRACT

Motherhood is a time accompanied by reductions in the neuroendocrine and emotional responses to stress as well as a resistance to stress-induced deficits in hippocampal-dependent learning. These changes may be mediated by structural plasticity in the hippocampus, a brain region that has been linked to stress, learning, and anxiety regulation. Dendritic spines, sites of excitatory synapses, are one aspect of hippocampal structure that may provide a cellular substrate for altered stress responsiveness during motherhood. To begin investigating this possibility, we examined the influence of stress on dendritic spines in the postpartum hippocampus. Virgin (i.e. female rats without reproductive experience) and postpartum female rats were subject to 20 min of inescapable swim stress and sacrificed 24 hr after stressor exposure along with unstressed controls. Brains were processed using Golgi impregnation and dendritic spine density analyzed on apical and basal dendrites of pyramidal neurons in the dorsal and ventral regions of area CA1 in the hippocampus. We show that in the ventral hippocampus, exposure to acute stress diminished dendritic spine density in virgin, but not postpartum, females. Acute stress had no effect on dendritic spines in the dorsal hippocampus of virgin or postpartum females, although mothers had more dendritic spines as compared to virgins. These data suggest that during motherhood, the hippocampus is resilient to the detrimental effects of stress which may contribute to other stress-related adaptations during this time.

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INTRODUCTION

Motherhood is widely acknowledged to be a time of both genuine joy and new experiences. It is also a time accompanied by many biological and behavioral modifications in the mother. Indeed, pregnancy, parturition (i.e. childbirth), and the postpartum period are characterized by dramatic hormonal changes in both rodents and humans (Rosenblatt 1994). Estrogen levels, which are high during late pregnancy, decline after parturition and remain at low levels thereafter. In contrast, progesterone begins to rise starting approximately 3–4 days after parturition to levels comparable to that during pregnancy, peaking between days 7 and 11 postpartum (Nelson, 2011). Levels of the adrenal steroid corticosterone also increase postpartum as do the neuropeptides oxytocin and prolactin (Voogt et.al., 1969; Slattery & Neumann, 2008). In addition to hormones, motherhood is typified by changes in experience. With the birth of offspring come added sights, sounds, tastes, as well as tactile and suckling stimulation. Thus, the presence of offspring provides the mother with an enriched sensory environment.

Complex interactions between endocrine and experiential events are responsible for the emergence of a number of behavioral modifications in mothers. Within moments of parturition, female rats that were previously unresponsive to pups engage in an elaborate repertoire of caregiving activities that include cleaning the pups, eating the debris of birth, nursing, nest building, licking and grooming, pup retrieval, assuming a nursing (i.e. arched back) posture over the pups, and increased aggression toward intruders (Kinsley et al., 2008; Leuner et al., 2010). In addition to these direct caregiving activities, a number of other behaviors may indirectly contribute to a mother's ability to care for her young. Certain cognitive abilities have been shown to be altered during pregnancy and the postpartum period including spatial navigation

abilities, objection recognition, attention, and behavioral flexibility (Darnaudéry et. al, 2007; Macbeth et al., 2008; Leuner et al, 2010; Leuner & Gould, 2010). Alterations in these types of cognitive behaviors may be particularly important in rodents who must leave the nest to forage for food and do so efficiently in order to return quickly to safeguard the offspring from predators (Kinsley et al., 2008). Additionally, successful parenting requires the maintenance of cognitive abilities while coping with the stresses and demands of parenting. In this regard, mothers have been shown to maintain their ability to perform associative learning tasks after stress exposure (Leuner & Shors, 2006). Although much less studied, the available evidence suggests that hormones and experience likewise contribute to cognitive changes at this time (Tomizawa et al., 2003; Macbeth & Luine, 2010; reviewed in Workman et. al, 2012).

Another well documented postpartum adaptation that occurs in rodent and human mothers involves altered emotional behavior including a reduction in anxiety. In rodents, it has been suggested that reduced anxiety-like behaviors may be useful for making the mother less fearful of her environment and subsequently more attentive to the needs and survival of her offspring (Macbeth & Luine, 2010). Similarly in humans, reduced anxiety can buffer against postpartum depression and other mental illnesses, which can be associated with inadequate childcare and subsequent poor cognitive and emotional development of the child (reviewed in Leuner et al., 2010). Like other postpartum behavioral adaptations, the attenuation in anxiety observed postpartum is driven by hormones and experience (George et al. 1987; reviewed in Lonstein, 2007). In this regard, oxytocin, prolactin, and environmental stimulation via contact with offspring stimulate anxiolysis (Torner et al., 2001; Amico et al., 2004; reviewed in Lonstein 2007; Neumann 2009).

The postpartum period is also a time characterized by alterations in the stress response. In virgins (i.e. those without reproductive or maternal experience), exposure to stressors activates the hypothalamic-pituitary-adrenal (HPA) axis which ultimately stimulates the release

of stress hormones (i.e glucocorticoids in rodents, cortisol in humans). In contrast, postpartum females, despite having elevated basal levels of stress hormones, show a diminished HPA response to a wide range of physical and psychological stressors (Shanks et al., 1999; Neumann 2003; Donner et. al, 2007). The mechanisms underlying altered endocrine stress responsiveness have been well characterized and involve inhibitory effects of oxytocin and prolactin on the HPA axis as well as reduced activity of pathways that stimulate the HPA axis (Slattery & Neumann, 2008).

There is a vast literature implicating the hippocampus in learning and memory processes (reviewed in Squire, 1992; Scoville & Milner, 2000; reviewed in Leuner & Gould, 2010). The hippocampus also plays significant but less well-known roles in the modulation of anxiety and regulatory control over the HPA axis (Fanselow & Dong, 2010; Leuner & Gould, 2010). New research has emerged which shows that the hippocampus can be divided into two zones based on connectivity and function: the rostral/dorsal zone and the caudal/ventral zone (Fanselow & Dong 2010). The dorsal hippocampus primarily projects onto cortical areas responsible for cognitive processing of visuospatial information and memory processes, as well as onto areas containing a high density of navigation-related neurons (Fanselow & Dong 2010). Functionally, dorsal hippocampus is responsible for cognitive abilities, such as declarative memory and spatial navigation (Fanselow & Dong 2010). On the other hand, the ventral hippocampus has been shown to be necessary for the display of anxiety behavior, through its projections to the amygdala and prefrontal cortex, regions implicated in emotions and stress regulation, and for the neuroendocrine response to stress through its projections to regions associated with the regulation of the HPA axis (Adhikari et al., 2010, Fanselow & Dong, 2010).

The hippocampus is a structure whose synaptic connections and dendrites undergo rearrangement throughout life (Leuner & Gould, 2010). One mechanism of synaptic plasticity involves alterations in dendritic spines, small protrusions found on the shaft of dendrites in the

mammalian brain on which 90% of excitatory synapses are formed (reviewed in Nimchinsky et al. 2002). These structures are regulated by a variety of hormones and experiences, including those associated with motherhood. For example, estrogen and environmental enrichment enhance complexity of dendrites and increase the numbers of dendritic spines (Woolley et al., 1990, Moser et al., 1994; Moser et al., 1997). Not surprisingly, motherhood itself also induces structural plasticity in the hippocampus including changes in dendritic spines, although the specific effects depend on a variety of factors including the postpartum time point and hippocampal sub-region examined (Leuner et al., 2010).

Previous experiments have demonstrated that in virgin females, acute stress diminishes dendritic spines in the CA1 region of the hippocampus (Shors et al. 2001, Leuner & Shors, 2004). Given that the postpartum period is associated with reduced stress responsiveness, we tested the hypothesis that dendritic spines in the postpartum hippocampus are resistant to the detrimental effects of stress. We analyzed the dorsal and ventral sub-regions separately in order to investigate the possibility that distinct sub-regions of the hippocampus may be differentially affected.

MATERIALS AND METHODS

Subjects

Age-matched adult virgin female and timed pregnant Sprague-Dawley purchased from Taconic (Germantown, NY) rats were used. Rats were housed individually on a 12 h/12 h light/dark cycle. On the day of birth (postpartum day 0, PD0), litters were culled to 10 pups. Rats were provided access to food and water *ad libitum* throughout the experiment. All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and conformed to the US NIH Guide for the Care and Use of Laboratory Animals.

Stressor exposure

This experiment consisted of 4 groups of rats (n = 6-8/group): 1) unstressed virgin females, 2) stressed virgin females, 3) unstressed postpartum females and, 4) stressed postpartum females. Twenty minutes of inescapable swimming was used as the stressor. For virgin females, vaginal smears were taken daily until the animal was in diestrus and then subjected to the forced swim procedure. Mothers were subjected to swim stress on PD8. 24 hr after the stressor, stressed rats were anesthetized with Euthasol along with unstressed virgins in proestrus and postpartum females on PD9 and the brains rapidly removed. .

Golgi impregnation

Golgi impregnation was accomplished using the FD Rapid Golgi Stain kit (FD Neurotechnologies; Ellicot City, MD). In brief, small blocks of tissue containing the hippocampus were incubated in a potassium dichromate, mercuric chloride, and potassium chromate solution (Solution A+B) for two weeks in the dark at room temperature. Brains were then transferred to solution C and stored at 4°C for 2 d. Next, coronal sections (150 µm) spanning the rostrocaudal extent of the hippocampus were cut on a Vibratome, mounted onto gelatinized slides, and dried at room temperature in the dark for 1-3 days. For development, slides were rinsed, incubated in solutions D + E for 10 min, dehydrated through a graded (50, 75, 95, 100 %) alcohol series (4 min each) with an extra 12 min rinse in 100% ethanol, cleared in xylene for 12 min, and coverslipped with Permount.

Microscopic analysis

All analyses were conducted blind to experimental conditions. Dendritic spine density was measured on Golgi impregnated pyramidal neurons of the CA1 region of the dorsal hippocampus (Anterior-posterior/AP -3.30mm, medial-lateral/ML 1.65 mm, dorsal-ventral/DV -3.43; Figure 1) and ventral hippocampus (AP -5.60 mm, ML 4.80 mm, DV 5.67 mm; Figure 1) using a Nikon 90i microscope and NIS Elements software. In each of these regions, dendritic

spine densities were analyzed on 4-5 fully impregnated neurons using a 100X oil objective. On selected neurons, dendritic spines were counted on 5 apical and 5 basal randomly selected dendritic segments (each 10-25 µm long). . Only spines extending away from the shaft were counted.

Behavioral analysis

A separate cohort of animals was used to assess behavioral performance in virgin and postpartum females during the swim stress procedure. This experiment consisted of 2 groups of rats: 1) virgin females in diestrus (n=7) and 2) postpartum females on PD8 (n=8). All animals were subjected to 20 min of inescapable swim stress and behavior was digitally recorded and scored later for the following: struggling (movements of the forelimbs, specifically the forepaws breaking above the surface and usually directed at the walls of the cylinder), swimming (paddling with forelimbs and hindlimbs, resulting in movement across at least two quadrants within 5 s), and immobility (making minimal movements necessary to keep head above water and rats remains in one quadrant).

Statistical analysis

Dendritic spine density was analyzed using two-way ANOVA. Behavioral data was analyzed using unpaired t-test. Neuman-Keuls post-hoc comparisons were applied when necessary.

RESULTS

Postpartum females are resistant to stress-induced dendritic spine loss

For CA1 pyramidal neurons in the ventral hippocampus (Figure 2), there was a significant interaction between stress and reproductive status on apical [$F(1,23)=24.03$, $P<0.0001$] and basal dendrites [$F(1,23)=23.59$, $P<0.0001$]. Stressed virgin females experienced

diminished spine density on both apical ($p<0.0001$) and basal ($p<0.0001$) dendrites in comparison to unstressed virgin females. In contrast, stressed mothers did not exhibit a change in dendritic spine density on apical or basal dendrites relative to their unstressed counterparts (P values > 0.05).

Unlike the ventral hippocampus, there was no significant interaction between reproductive status and stress on spine density of apical [$F(1,24)=0.12$, $P=0.73$] or basal dendrites [$F(1,24)=0.42$,$P=0.52$] in the dorsal CA1 region of the hippocampus (Figure 3). However, there was a significant main effect of reproductive status on apical dendrites [$F(1, 24)=5.86$, $P=0.02$] such that mothers had a greater density of dendritic spines as compared to virgins.

Postpartum females behave similarly to virgins during forced swim stress

During the 20 min swim stress, mothers did not show a significant difference in time spent swimming ($t(13)=0.23$, $p=0.30$) in comparison to virgin females (Figure 4), Similarly, virgin and postpartum females time equivalent amounts of time struggling ($t(13)=0.18$, $p=0.32$) and immobile ($t(13)=0.34$, $p=0.90$) during the swim stress (Figure 4).

DISCUSSION

Here we show that during motherhood, dendritic spines in ventral hippocampus are resistant to the effects of acute stress. One day after acute swim stress exposure, dendritic spine density on apical and basal dendrites of the CA1 ventral hippocampus was significantly diminished in virgin females; this reduction was not present in postpartum females. On the other hand, acute stress had no effect on dendritic spines in the dorsal hippocampus of virgin or postpartum females. However, it is interesting to note that mothers possessed a larger dendritic spine concentration on apical dendrites than virgin females in this region. Together, these data

reveal that postpartum females experience adaptive neuroanatomical plasticity which is perhaps crucial in reduced anxiety and other alterations in stress responsiveness typical of motherhood.

One possible explanation for why mothers do not experience dendritic spine loss in the ventral hippocampus after acute stress like virgins do may be because they experience the stress differently. Although we cannot completely exclude this possibility, we found that virgin and postpartum females spent comparable amounts of time engaging in swimming, struggling, and immobility during the stress paradigm. Therefore, it can be reasonably assumed virgin and postpartum females experience acute swim stress similarly.

As described earlier, recent literature treats the hippocampus not as a homogenous structure but rather as two unique zones, the dorsal and the ventral poles. The ventral hippocampus is connected with areas such as the amygdala and hypothalamus which support its role in anxiety and negative feedback regulation of the HPA axis (reviewed in Fanselow & Dong, 2010). Since motherhood is associated with hypo-responsiveness of the HPA axis to stress and attenuated anxiety (reviewed in Lonstein 2007; reviewed in Macbeth & Luine, 2010), resilience of dendritic spines in the ventral hippocampus to acute stress might be a potential adaptive neural mechanism which allows the mother to cope with the general stress and adverse challenges accompanying motherhood.

Previous research demonstrated that in virgin females, dendritic spines in the dorsal CA1 region of the hippocampus are susceptible to the effects of acute stress, although the ventral region was not examined (Shors et al., 2001). A possible explanation for why we did not detect stress effects on dendritic spines in the dorsal hippocampus may be related to the different acute stressors employed - 20 min of swim stress here versus 30 min of restraint and inescapable tail shock previously. Dorsal CA1 hippocampus has a higher density of place cells which encode spatial location and projects onto navigation neurons in areas necessary for environmental exploration (Fanselow & Dong, 2010). The stress of being restrained and unable

to move could cause the dorsal hippocampus and its related structures to be impaired due to the nature of the paradigm. On the other hand, inescapable swim stress still allows for the animal to move freely and dorsal hippocampus remains engaged. Nonetheless, we did observe that mothers had more dendritic spines in the dorsal CA1 region of the hippocampus as compared to virgins which may account for their superior spatial navigation abilities (Tomizawa et al., 2003, Pawluski et al., 2006, Darnaudéry et. al, 2007). Future studies examining how both the dorsal and ventral hippocampus are affected by different stressor paradigms need to be conducted in order to better understand the effects of acute stress on these distinct sub-regions of the hippocampus. One possibility could be an extension of the Shors et.al (2001) experiment to analyze the effects of acute restraint stress on dendritic spines in both dorsal and ventral hippocampus. Given that the ventral hippocampus and its projections are critically involved in regulating the neuroendocrine response to stress, it is likely that the negative impact of acute stress on dendritic spines would also be present on the ventral region (Radley & Sawchenko, 2011).

The underlying mechanism by which motherhood protects dendritic spines in the ventral hippocampus from the negative actions of acute stress have yet to be determined but are likely to involve hormonal and experiential changes associated with this time. For instance, the prolactin and oxytocin systems are up-regulated postpartum (Nelson, 2011). Both of these hormones have been implicated in hippocampal plasticity and are known to buffer the brain against the negative actions of stress hormones which are elevated during the postpartum period (Tomizawa et al. 2003; Neumann 2009; Numan and Woodside, 2010). Motherhood could also exert a protective effect on structural plasticity via infant contact which regulates a number of postpartum-related adaptations including reduced anxiety (reviewed in Lonstein, 2007). Infant contact and other stimuli from the offspring may represent an enriched environment which can have beneficial effects on hippocampal structure and protect against the negative actions of stress and stress hormones (Hutchinson et al., 2012).

Mothers undergo many adaptive changes to help them adapt to new experiences but this can also be tremendously stressful on the body and brain. For instance, the sudden withdrawal of pregnancy hormones upon parturition activates neural and endocrine circuits necessary for maternal behaviors, but might also play a role in the onset of postpartum mood disorders (Brunton & Russell 2008). This study reveals a better understanding of hippocampal plasticity in response to stress. Stress-induced alterations in hippocampal structure, including dendritic spines, have been implicated in mood disorders (Leuner & Shors, 2012). Thus, this research could lead to a better understanding of postpartum depression and mental health issues which are experienced by approximately 15% of new mothers. Identifying the conditions under which the postpartum brain is resilient to stress could lead to a more effective treatment. This is crucial not only for the mental health of the mother, but for the health of the offspring as well since the emotional well-being of the mother influences her reactions and nurturing of the offspring. Thus, an understanding of the brain during motherhood is crucial for the health of the offspring as well as the health of the mother.

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APPENDIX

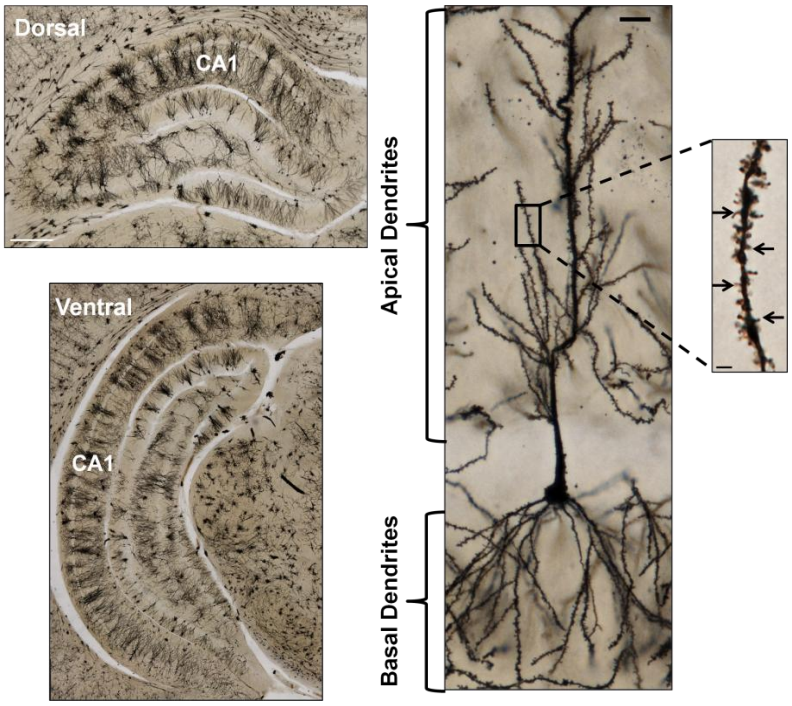

Figure 1.

Left: Golgi-stained sections of dorsal and ventral hippocampus showing the CA1 region (scale bar = 500 µm).

Right: representative CA1 pyramidal cell (scale bar = 20 µm) and magnified dendritic segment (scale bar = 2 µm). Arrows indicate dendritic spines.

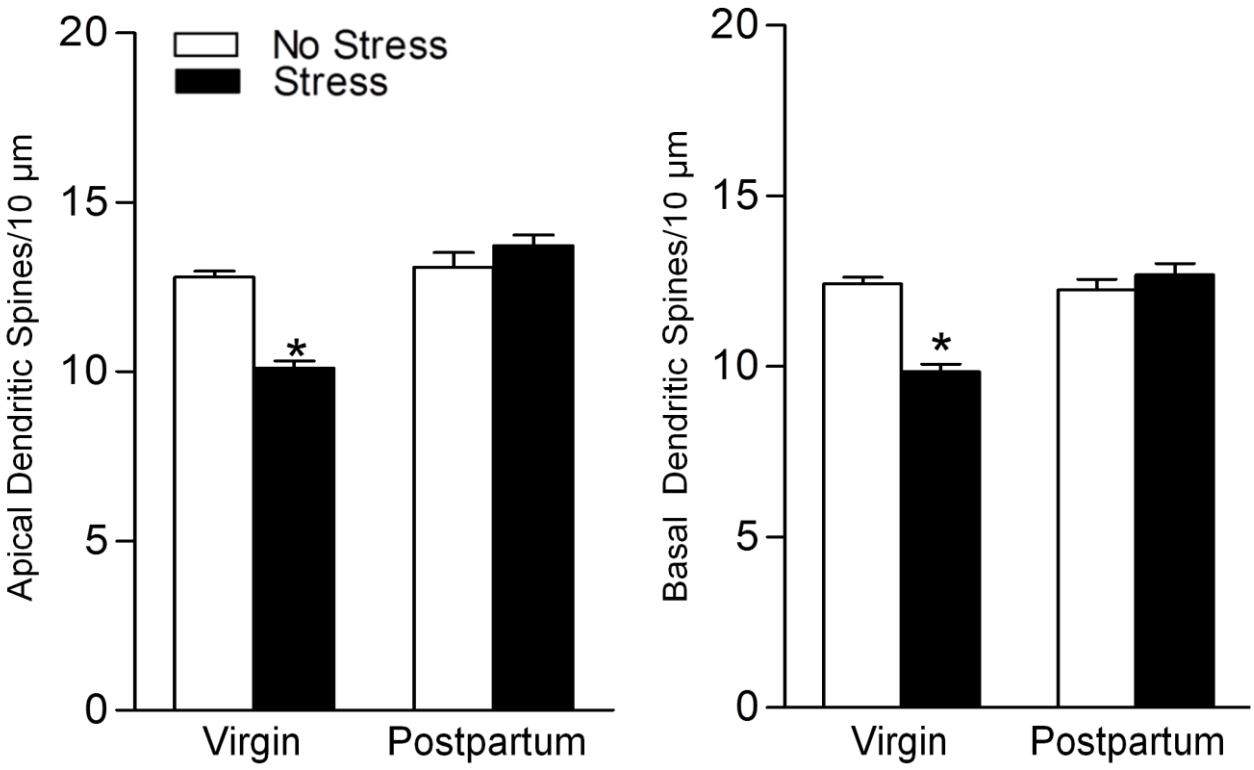

Figure 2.

In virgin females, acute stress diminished spine density on apical and basal dendrites of CA1 pyramidal neurons in the ventral hippocampus. In contrast, dendritic spine density was unaffected by acute stress in postpartum females. *p < 0.05.

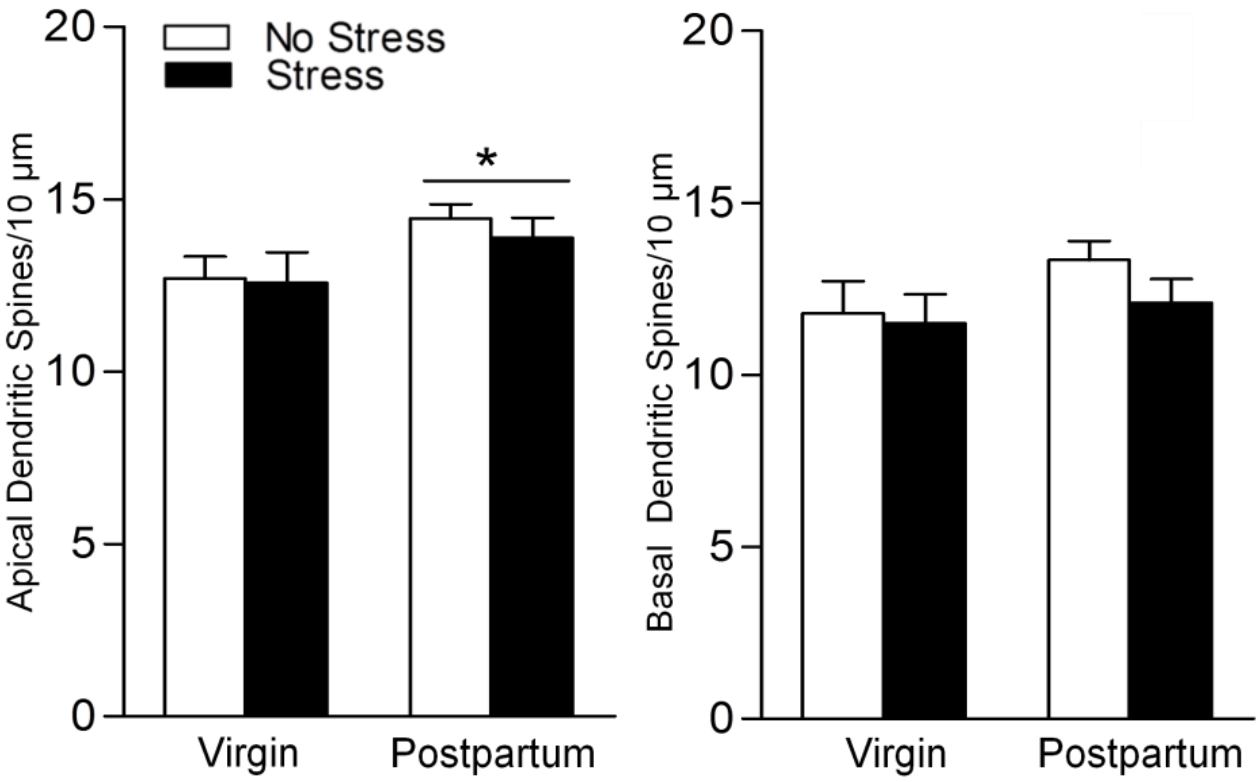

Figure 3. Although mothers had a greater density of spines on apical dendrites of CA1 pyramidal neurons in the dorsal hippocampus compared to virgins, there were no effects of stress and no significant interaction between stress and reproductive status. Spine density on basal dendrites did not differ among groups. *p < 0.05.

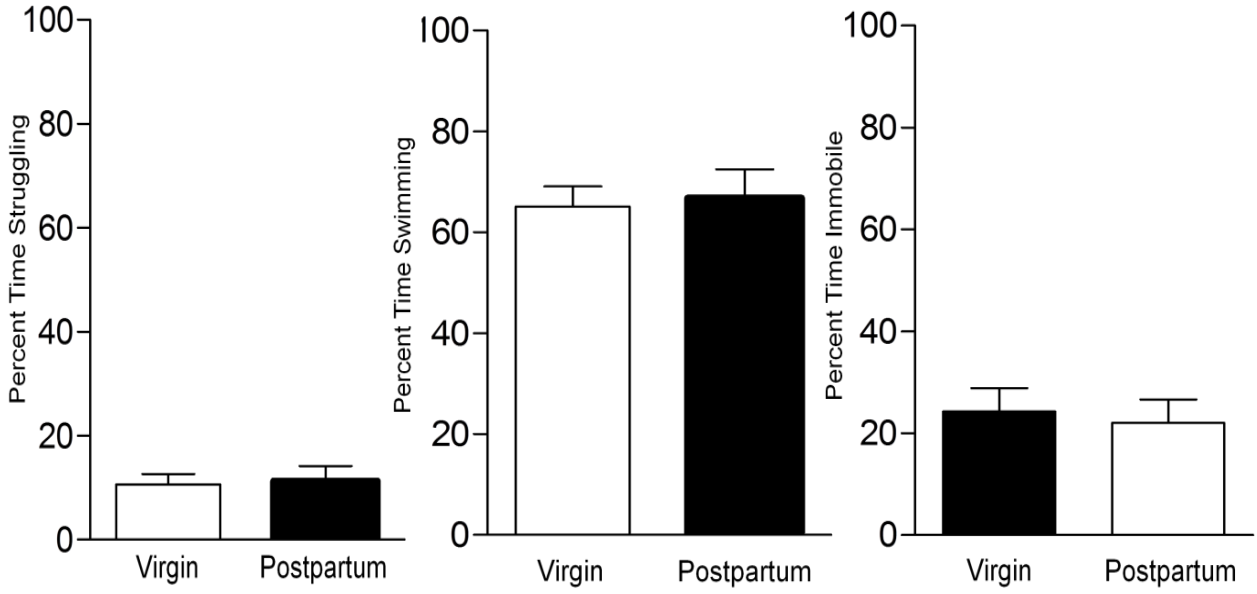

Figure 4. The percentage of time engaged in struggling, swimming, and immobility during the acute swim stress procedure did not differ between virgin and postpartum females.